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Relationship of Campylobacter Isolated from Poultry and from Darkling Beetles in New Zealand

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SUMMARY. *Campylobacter*, a foodborne pathogen closely associated with poultry, is considered to be an important agent of human gastroenteritis in New Zealand. The pathways involved in the contamination of poultry flocks remain unclear; however, many vectors, such as insects, rodents, and wild birds, have been implicated. Infestation of poultry houses by insects, particularly darkling beetles (*Alphitobius diaperinus*), is difficult to control. Furthermore, darkling beetles are known vectors for a variety of pathogens that include *Salmonella*, infectious bursal disease virus, *Aspergillus*, *Escherichia coli*, and Marek's disease virus. In this investigation, the relationship between darkling beetles and *Campylobacter* contamination of poultry flocks was investigated. A New Zealand breeder flock and four of its progeny broiler flocks were included in the study. Samples of beetles and of intestinal excreta of the birds were cultured for the presence of *Campylobacter* spp. A subset of the recovered isolates was subsequently genotyped using *flaA* short variable region (SVR) DNA sequence analysis. A large number of *Campylobacter* subtypes were isolated, indicating that *Campylobacter* colonization of poultry is likely to arise from a number of different reservoirs. However, a set of genetically distinct isolates were found to be common to the broiler flocks and to the beetles. This research provides data that indicates that *Alphitobius diaperinus* may serve as a source of *Campylobacter* contamination of poultry. A more thorough understanding of the relationship between beetle infestation and the *Campylobacter* status of poultry flocks should enable progress in further development of biosecurity control measures.

RESUMEN. Relación entre un aislamiento de Campylobacter obtenido a partir de aves con el obtenido a partir de escarabajos de la cama en Nueva Zelanda.

El *Campylobacter*, bacteria patógena transmitida en el alimento y asociada con la producción avícola, es considerado como un agente importante de la gastroenteritis humana en Nueva Zelanda. Las vías involucradas en la contaminación de los lotes de aves no son claras, sin embargo, han sido implicados muchos vectores tales como los insectos, roedores y aves salvajes. La infestación de galpones por insectos, en particular por el escarabajo de la cama (*Alphitobius diaperinus*), es difícil de controlar. El escarabajo de la cama es un vector conocido de una variedad de patógenos que incluyen *Salmonella*, virus de la enfermedad infecciosa de la bolsa, *Aspergillus*, *Escherichia coli* y virus de la enfermedad de Marek. Se investigó la relación existente entre el escarabajo de la cama y la contaminación por *Campylobacter* en lotes de aves. Se incluyeron un lote de reproductoras de engorde de Nueva Zelanda y cuatro de sus progenies. Se tomaron y se cultivaron muestras de escarabajos de la cama y de la excreta intestinal de las aves con el fin de determinar la presencia de *Campylobacter* spp. Se realizó el estudio genotípico de un subgrupo de los aislamientos obtenidos mediante el análisis de la secuencia de ADN perteneciente a la región variable corta *fla*A. Se aisló un gran número de subtipos de *Campylobacter*, indicando que la colonización por *Campylobacter* en las aves es ocasionada probablemente por diferentes reservorios. Sin embargo, se encontró que un grupo de aislamientos genéticamente diferentes eran similares a los encontrados en lotes de pollos de engorde y en escarabajos de la cama. Se suministran datos que indican que el *Alphitobius diaperinus* puede servir como una fuente de contaminación de las aves con *Campylobacter*. Un mayor conocimiento de la relación existente entre la infestación de los escarabajos de la cama y el estatus de *Campylobacter* en los lotes de aves permitirá obtener

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mayores progresos en el desarrollo de medidas de bioseguridad para el control de la infección por *Campylobacter*.

Key words: *Campylobacter*, darkling beetle (*Alphitobius diaperinus*), epidemiology, DNA sequence analysis

Abbreviations: L = liter; SVR = short variable region

Campylobacter jejuni, a gram-negative, microaerophilic bacteria, is presently believed to be the leading bacterial etiologic agent of acute gastroenteritis in the human population; the total number of *Campylobacter* enteritis cases in the United States is estimated at 2.4 million per year, or approximately 1%–2% of the population per year (4,32,35,36). Similar rates have also been reported for Europe and the United Kingdom (31). New Zealand has an incidence rate that is three to four times that reported in other developed countries (244.5 cases per 100,000 population per annum, with a peak of 320 per 100,000 population in 1998) (26). Gastroenteritis caused by *Campylobacter* can have an incubation period of 1 to 10 days; however, most cases have an incubation period of 3 to 5 days. Symptoms include profuse diarrhea, which may be bloody, abdominal pain, fever, headache, and malaise. Infection is usually self limiting, resolving within a week, although excretion of the organism in feces may continue for several weeks (4,5,8,38). Relative to other enteropathogens, *Campylobacter* has a low infective dose of 500 organisms; *Salmonella* requires greater than 10^5 organisms (2). Serious but infrequent complications to acute campylobacteriosis can arise and include hemolytic uremic syndrome, hepatitis, and Guillain–Barré Syndrome (1,13,38).

Handling and consumption of poultry or poultry-related products are considered to be primary sources for *Campylobacter*-induced disease in humans (7,18,27). A national survey conducted in 1995 by the New Zealand Ministry of Health revealed that 52% (82/159) of raw poultry samples tested were positive for *Campylobacter* (10). However, the pathways involved in *Campylobacter* contamination of poultry flocks remain unclear. Several suspected sources or vectors of contamination have been studied and include feed, drinking water, farm surface water, wild birds, rodents, insects, air, domestic pets, and farm animals (11,14,16,17,19,28,29). Biosecurity practices are considered important in minimizing contamination of poultry flocks by *Campylobacter* (11,14,15,20). However, even with implementation of the most stringent hygiene measures, it is difficult to avoid infestation of the shed or house by insects. One insect that is particularly difficult to control is the darkling beetle (*Alphitobius diaperinus*), also known as the lesser mealworm.

Darkling beetles are known vectors for a variety of pathogens that include *Salmonella*, infectious bursal disease virus, *Aspergillus*, *Escherichia coli*, and Marek's disease virus (9,12,21,22,23). In addition to its disease association, the darkling beetle is also of significance because of its ability to cause damage to poultry house insulation, its consumption of feed intended for the poultry, and its status as an urban pest. Conditions in modern poultry houses are ideal for the rapid development of darkling beetle populations; levels of infestation can reach up to 1000 beetles per square meter (3,33). In an effort to investigate the relationship between *Campylobacter* contamination of poultry flocks and the presence of darkling beetles, a breeder flock and four related broiler flocks in New Zealand were studied. Poultry-associated samples, beetles, larvae, and environmental samples were collected, cultured for *Campylobacter,* and genotyped to determine the relationships between the isolates (24). Determination of the role of darkling beetles in *Campylobacter* contamination of poultry will provide information that will facilitate the development of targeted intervention strategies. These interventions will facilitate the delivery of pathogen-free birds to the abattoir and, consequently, should reduce the incidence of human exposure.

MATERIALS AND METHODS

Beetle traps. Beetle presence was sampled using Arends tube traps prepared as described by Safrit and Axtell (30). Briefly, tube traps comprised a rolled piece of corrugated cardboard (23.5 cm long × 22.5 cm wide) inserted into a piece of rigid polyvinylchloride pipe (25 cm long × 3.8 cm wide).

Breeder flocks. This investigation was performed in cooperation with one of the two breeder production companies serving the New Zealand poultry industry. A description of New Zealand husbandry practices is provided below. One-day-old chicks (5903 females and 818 males) hatched from the grandparent flock were placed into one of six houses on a rearing site coded as

site "X." Each of the six houses on rearing site X contained one parent flock. At 18 wk of age, the parent flock was transferred to a parent production site, where the fertile eggs produced were hatched as broilers for the retail market. The X6 rearing house size was 584 m^2, with heating provided by gas brooders and ventilation by six wall fans. Apart from drinkers and brooders, the only other pieces of equipment present were corner barriers and perch stands (employed in training for nestbox use during production). The total mortality during the rearing period for the X6 flock was 4.8%. The average bird weight, when transferred to the production site "Y" at 18 wk of age, was 1.86 kg.

A concrete pad was installed at the main entrance of the X6 house to facilitate clean-out procedures (decontaminated at flock depletion). The house was constructed of timber panels with a corrugated iron roof; the floor was concrete. All staff and visitors showered on site and adhered to any company-specific entry requirements. Disinfectant footbaths were provided for use at the exit of the lunch room/shower block, at the entrance to each shed annex or control room, and again before direct entry into the bird pens. Black boots were worn outside of the house; white boots were worn inside the house.

Breeder birds received several vaccinations, as indicated by the company veterinarian, for protection against endemic poultry diseases. Additionally, the flock was medicated twice for coccidiosis using Totraurazole, a coccidiocide that has no bacterial action (and therefore should not affect the flock's *Campylobacter* status). The birds were fed manually once a day according to the nutritional guidelines provided by the Pedigree breeding company. Bell drinkers were supplemented by minidrinkers. Water was obtained from a bore supply that was chlorinated to give a level of 0.2 ppm free available chlorine.

Egg production commenced at approximately 20 wk of age; the eggs were harvested for hatching purposes when they reached a minimum weight of 50 g, at approximately 23 wk of age. Eggs were collected four times a day. The nestboxes were maintained by replacing the nest litter with fresh shavings every 2 wk and adding 20 g per nest of formaldehyde prills. Any visible fecal soiling in the nest was removed by the house operators. House operators sanitized their hands prior to egg collection. Floor eggs were collected last, stored on a separate filler, and were not used for hatching purposes. Within 3 h of collection, the eggs were fumigated with formaldehyde gas and stored at 16 C, with 70% relative humidity, until required for hatching.

Study populations. One study population (age: day of hatch to 18 wk) was reared on a parent stock rearing site, referred to as X, in shed X6. Twenty-three Arends tube traps were equally spaced throughout the house. The tube traps were secured to side walls using insulation tape and plastic electrical ties.

Biosecurity and hygiene measures for rearing shed X6 were recorded as follows. Two days after removal of the previous flock, the shed was cleaned using a strong disinfectant agent (FarmFluid S, Antec International, Sudbury, United Kingdom) followed by terminal disinfection using QCT (Qualtech, Inc., Hamilton, New Zealand) and Triton (Qualtech, Inc.). FarmFluid S is a blend of organic acids, surfactants, and high– and low–molecular weight biocides. QCT is a quat and glutaraldehyde disinfectant, whereas Triton is a surfactant. Surface sample swabs were collected for laboratory evaluation to ensure the farm was in compliance with the company standard for hygiene and negative *Salmonella* status. One week later, an insecticide containing cypermethrin (Ripcord, Bayer, Leverkusen, Germany) was applied to the shed surfaces and equipment at a rate of 2 liters per 400 liters of water. Rodents were controlled through bait stations loaded with Pestoff rodent blocks (Fumapest, Auckland, New Zealand) (active ingredient: 20 ppm brodifacoum). Bait stations were monitored and replenished weekly. Wild birds did not have access to the sheds, and farm animals were not grazed within a minimum of 3 m of the shed surrounds. All staff and visitors were required to shower on site. Disinfectant footbaths were provided for use at the entrance to each shed annex and before direct entry into the bird pens. One-day-old chicks were used to populate the parent stock rearing site.

The second study population (age: 18–45 wk) was reared on a parent stock production site, referred to as Y, in shed Y1. The birds in this study population were the same birds as those evaluated at the parent stock rearing site above. Twenty-one Arends tube traps were placed in the center area of the house near the nestbox stands in shed Y1. This area was chosen because the litter was loosely packed, which is more favored by beetles (30). The 21 tube traps were equally divided between the seven pens within the Y1 shed. The tube traps in this facility were secured to side walls and to nestbox stands using insulation tape and plastic electrical ties. Biosecurity and hygiene measures were the same as for the parent stock rearing site described previously. Twenty fresh fecal and/or cecal droppings were randomly collected from the houses and pooled weekly during breeder flock production.

Broiler flocks. A total of four broiler flocks were monitored during this investigation. Two flocks each were raised in either of two sheds (Q1 and Q2) on farm Q. Flocks were referred to as Q1-1 (shed Q1, flock 1), Q1-2 (shed Q1, flock 2), Q2-1 (shed Q2, flock 1), and Q2-2 (shed Q2, flock 2). The broiler sheds were observed to be heavily infested with beetles; therefore, only five Arends tube traps were used to monitor beetle presence within each broiler flock. The five tube traps were randomly placed throughout each of the sheds tested. Twenty fresh fecal and/or cecal droppings were randomly collected and pooled weekly during broiler flock production from each flock. The broiler rearing facility followed a more relaxed

biosecurity regime relative to the breeder rearing and breeder production facilities. Showering on site was not required. No farm animals were grazed directly around the sheds. A rodent monitoring program was in place, as were disinfectant footbaths at the shed entrance.

Environmental monitoring. For the breeder rearing and production facilities, the temperature and relative humidity were monitored on a continual basis (every 20 min) by a BoxCar® Pro logger (Onset Computer, Bourne, MA). Collected data was downloaded and exported to a Lotus spreadsheet for analysis. For the broiler facility, the temperature was monitored using a hand-held temperature probe. Collected data were then confirmed against the grower's in-house temperature monitoring equipment.

Environmental sampling. Observed risks to biosecurity for each flock tested were noted, and where possible, a sample was collected to test for *Campylobacter* presence. Examples of observed risks included droppings from other animals (goats, cows, and pukekos), insects other than darkling beetles found in tube traps, and creek water.

Cultural analysis. *Beetles/insects.* After sampling, traps were individually collected into sterile ziplock plastic bags. Sample bags were flushed with CO_2 to anesthetize the beetles and larvae, which were subsequently extracted from the cardboard insert by shaking the trap directly into the ziplock bag. The cardboard was subsequently unrolled to check for any remaining insects. A rubber mallet was used to gently crush the specimens so as to expose the internal contents. Bolton's enrichment broth (Oxoid, Becton Dickinson, Sparks, MD) was added to the crushed sample, such that each sample was completely covered. The suspension was enriched at 42 C for 48 hr, after which 0.1 ml of the enriched suspension was plated onto Campy–Cefex agar plates and incubated at 42 C for 36–48 hr in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2) using BBL gaspak jars and BBL Campypak Plus™ microaerophilic gas-generating envelopes (Oxoid, Becton Dickinson). Following incubation, a representative number of presumptive *Campylobacter* colonies were confirmed by observation of typical cellular morphology using phase contrast microscopy and with a commercial latex agglutination kit (Integrated Diagnostics, Inc., Baltimore, MD).

Poultry cecal/fecal droppings/other animal droppings. Pooled samples were weighed into sterile stomacher bags and diluted 1:3 (w/v) with Difco buffered peptone water (BPW; Becton Dickinson). A 10-µl loopful of suspension was plated onto Campy–Cefex and incubated as previously described. Following incubation, a representative number of presumptive *Campylobacter* colonies were confirmed by observation of typical cellular morphology using phase contrast microscopy and with a commercial latex agglutination kit (Integrated Diagnostics, Inc.).

Creek-water samples. Water samples were treated as previously described (6). Briefly, approximately 180 ml of sampled water was filtered through a 0.45-µm filter. Filters were aseptically transferred to 25 ml of Bolton's enrichment broth, enriched, and plated as described above. Presumptive *Campylobacter* colonies were identified as described above.

Molecular subtype analysis. One *Campylobacter* isolate, originating from each positive sample type during each sampling period, was chosen for subtype analysis. Isolated colonies of *Campylobacter* were resuspended in 300 µl of sterile H_2O and held at 100 C for 10 min. Ten microliters of each boiled cell suspension was used as template for *flaA* short variable region polymerase chain reaction (*flaA* SVR PCR) with the following primers: FLA242FU: 5′CTA TGG ATG AGC AAT TWA AAA T3′ and FLA625RU: 5′CAA GWC CTG TTC CWA CTG AAG3′ (24). A 35-cycle reaction was used with 1 min denaturing at 96 C, 1 min annealing at 52 C, and a 1 min extension at 72 C. The resulting product was approximately 425 base pairs (bp) in length. Sequence data were generated using either the FLA242FU primer or the FLA625RU primer with the Big-Dye Dye-Terminator Cycle Sequencing Kit (ABI-PE, Foster City, CA). Data was assembled with Sequencher 4.1 (GeneCodes Corp., Ann Arbor, MI) and aligned using ClustalX (37). Aligned sequences were compared and dendograms generated using the UPGMA algorithm with absolute distance measurements in PAUP*4.0 (Phylogentic Analysis Using Parsimony) (34).

RESULTS

Breeder rearing population. No beetles were recovered during the 18-wk study period; however, it should be noted that insecticide was applied to the shed surfaces prior to chick placement. At the end of the rearing period, approximately 20 liters of floor litter was removed and held at the laboratory. A sample of the litter was tested weekly for 2 mo for beetle presence; no beetles were detected.

Fecal samples, collected weekly, were positive for *Campylobacter* in the breeder rearing flock from 14 to 17 wk of age. Additional environmental samples, thought to be relevant to biosecurity, were also tested for presence of *Campylobacter*. These samples included a dead rat, an earwig, and fecal droppings from other poultry flocks on the same farm. Information on collected isolates is presented in Table 1. Only the poultry fecal droppings were positive for *Campylobacter;* both the rat and the earwig samples were culturally negative for *Campylobacter*.

Breeder production population. The flock in this study population was the same flock as the one evaluated at the breeder rearing population

Table 1. *Campylobacter* isolates recovered and subtyped in this investigation.

Farm	Isolate designation	Source of isolate	Recovery date
Breeder rearing farm X (shed X6)			
	X6-14F	Poultry fecal dropping (14 wk)	May 29, 2000
	X6-15F(A)	Poultry fecal dropping (15 wk)	June 07, 2000
	X6-15F(B)	Poultry fecal dropping (15 wk)	June 07, 2000
	X6-16F	Poultry fecal dropping (16 wk)	June 12, 2000
	X6-17F(A)	Poultry fecal dropping (17 wk)	June 19, 2000
	X6-17F(B)	Poultry fecal dropping (17 wk)	June 19, 2000
	X3/X4F	Other poultry fecal dropping (20 wk)	February 28, 2000
	X2F	Other poultry fecal dropping	May 29, 2000
Breeder production farm Y (shed Y1)			
	Y1-19F(A)	Poultry fecal dropping (19 wk)	July 03, 2000
	Y1-19F(B)	Poultry fecal dropping (19 wk)	July 03, 2000
	Y1-28F(A)	Poultry fecal dropping (28 wk)	September 04, 2000
	Y1-28F(B)	Poultry fecal dropping (28 wk)	September 04, 2000
	Y1-28F(C)	Poultry fecal dropping (28 wk)	September 04, 2000
	Y1-40F(A)	Poultry fecal dropping (40 wk)	November 27, 2000
	Y1-40F(B)	Poultry fecal dropping (40 wk)	November 27, 2000
	Y1-40F(C)	Poultry fecal dropping (40 wk)	November 27, 2000
	Y1-40F(D)	Poultry fecal dropping (40 wk)	November 27, 2000
	Y1-43F(A)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(B)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(C)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(D)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(E)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(F)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(G)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(H)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-43F(I)	Poultry fecal dropping (43 wk)	December 18, 2000
	Y1-45F(A)	Poultry fecal dropping (45 wk)	January 03, 2001
	Y1-45F(B)	Poultry fecal dropping (45 wk)	January 03, 2001
	Y7F(A)	Other poultry fecal dropping from infested shed	June 23, 2000
	Y7F(B)	Other poultry fecal dropping from infested shed	June 23, 2000
	YP(A)	Pukeko dropping	November 27, 2000
	YP(B)	Pukeko dropping	January 03, 2001
	YW	Creek water	January 03, 2001
	Y1-58B(A)	Beetles (58 wk)	April 02, 2001
	Y1-58B(B)	Beetles (58 wk)	April 02, 2001
Broiler production farm Q, Q1-1 and Q2-1			
	Q2-1F	Poultry fecal dropping flock Q2-1 (4 wk)	October 20, 2000
	Q1-1B(A)	Beetles (6 wk)	October 26, 2000
	Q1-1B(B)	Beetles (6 wk)	October 26, 2000
	Q1-1B(C)	Beetles (6 wk)	October 26, 2000
	Q1-1B(D)	Beetles (6 wk)	October 26, 2000
	Q2-1B(A)	Beetles (6 wk)	November 30, 2000
	Q2-1B(B)	Beetles (6 wk)	November 30, 2000
	Q2-1B(C)	Beetles (6 wk)	November 30, 2000
	Q2-1B(D)	Beetles (6 wk)	November 30, 2000
Broiler production farm Q, Q1-2 and Q2-2			
	Q2-2F(A)	Poultry fecal dropping flock Q2-2 (3 wk)	February 14, 2001
	Q2-2F(B)	Poultry fecal dropping flock Q2-2 (4 wk)	February 21, 2001

Table 1. Continued.

Farm	Isolate designation	Source of isolate	Recovery date
	Q2-2B(A)	Beetles (3 wk)	February 14, 2001
	Q2-2B(B)	Beetles (4 wk)	February 21, 2001
	Q2-2B(C)	Beetles (4 wk)	February 21, 2001
	Q2-2B(D)	Beetles (4 wk)	February 21, 2001

study above. Beetles were recovered from seven of the samples taken; flock ages were 22 wk, 23 wk, 25 wk, 28 wk, 33 wk, 40 wk, and 58 wk. Only the final beetle positive sample (flock age 58 wk) was *Campylobacter* positive.

Fecal samples collected at flock ages 19 wk through 45 wk were all positive for *Campylobacter*. Information on collected and subtyped isolates is presented in Table 1. Additional environmental samples, thought to be relevant to biosecurity, were also tested for presence of *Campylobacter*. These samples included fecal droppings from pukekos (*Porphyrio porphyrio melanotus*), fecal droppings from a goat, fecal droppings from a cow, creek water, fecal droppings from other poultry flocks on the same farm, and beetles collected within another shed (shed Y7) on the same farm. Information on collected isolates is presented in Table 1. The pukeko droppings, the creek water, the poultry fecal droppings, and the beetles collected from another shed (shed Y7) were positive for *Campylobacter*; the goat and the cow samples were culturally negative for *Campylobacter*.

Broiler population. A total of four broiler flocks were monitored during this investigation. Two flocks each were raised in either of two sheds (Q1 and Q2) on farm Q. Flocks were referred to as Q1-1 (shed Q1, flock 1), Q1-2 (shed Q1, flock 2), Q2-1 (shed Q2, flock 1), and Q2-2 (shed Q2, flock 2). Beetles were recovered in all four flocks tested. Beetles were positive for *Campylobacter* in three of the flocks tested; beetles collected from flock Q1-2 were *Campylobacter* negative. Information on collected and subtyped isolates is presented in Table 1. Twenty fecal and/or cecal droppings were collected and pooled weekly during broiler flock production from each flock. All four flocks were positive for *Campylobacter* by fecal sampling. Information on collected isolates is presented in Table 1.

Molecular subtype analyses of *Campylobacter* isolates. Molecular subtype analyses of *Campylobacter* isolated from fecal samples within individual flocks revealed that multiple clones of *Campylobacter* could be present within a single flock. As many as seven distinct clones were isolated from the breeder production farm Y flock (Fig. 1) over the 26-wk monitoring period. Additionally, genotype analysis demonstrated that similar clones were present within the same flock when located at two different facilities, farm X and farm Y. It should be noted that these clones [all X6 isolates, Y1-19F(A) 07/03/2000, and Y1-19F(B) 07/03/2000] were present shortly before and after the birds were moved from the breeder rearing facility to the breeder production facility. This clone was not detected during the later stages of farm Y sampling.

Four *Campylobacter* isolates originating from feces of poultry flocks raised contemporaneously on the same farms were analyzed. Two isolates, X3/X4F and X2F, were obtained from farm X during the early stages of sampling. Genotype analysis revealed that these isolates were distinct from one another as well as distinct from the isolates obtained from the farm X flock under investigation. However, the two types obtained from X3/X4F and X2F were observed in poultry feces from the farm Y flock. The remaining two isolates, Y7F(A) and Y7F(B), were obtained from poultry feces from farm Y prior to placement of the investigated flock. Genotype analysis revealed that these two isolates, Y7F(A) and Y7F(B), were distinct from one another. However, each of the subtypes was obtained during investigation of the farm Y flock.

Beetle samples positive for *Campylobacter* were isolated from four of the six flocks monitored. Subtype analysis on *Campylobacter* isolated from the breeder production farm Y beetles revealed two distinct clones, Y1-58B(A) and Y1-58B(B). Both of these clones were present in the feces from the flock at earlier sampling dates. Overall analysis of *Campylobacter* isolates obtained from broiler flock beetles revealed two distinct subtypes circulating through the broiler farms. *Campylobacter* isolates were obtained from both fecal and beetle samples on farm Q2-2. The *Campylobacter* isolate obtained from poultry feces prior to those obtained from beetles were the same subtype as the isolates recovered from the beetles. The fecal isolate

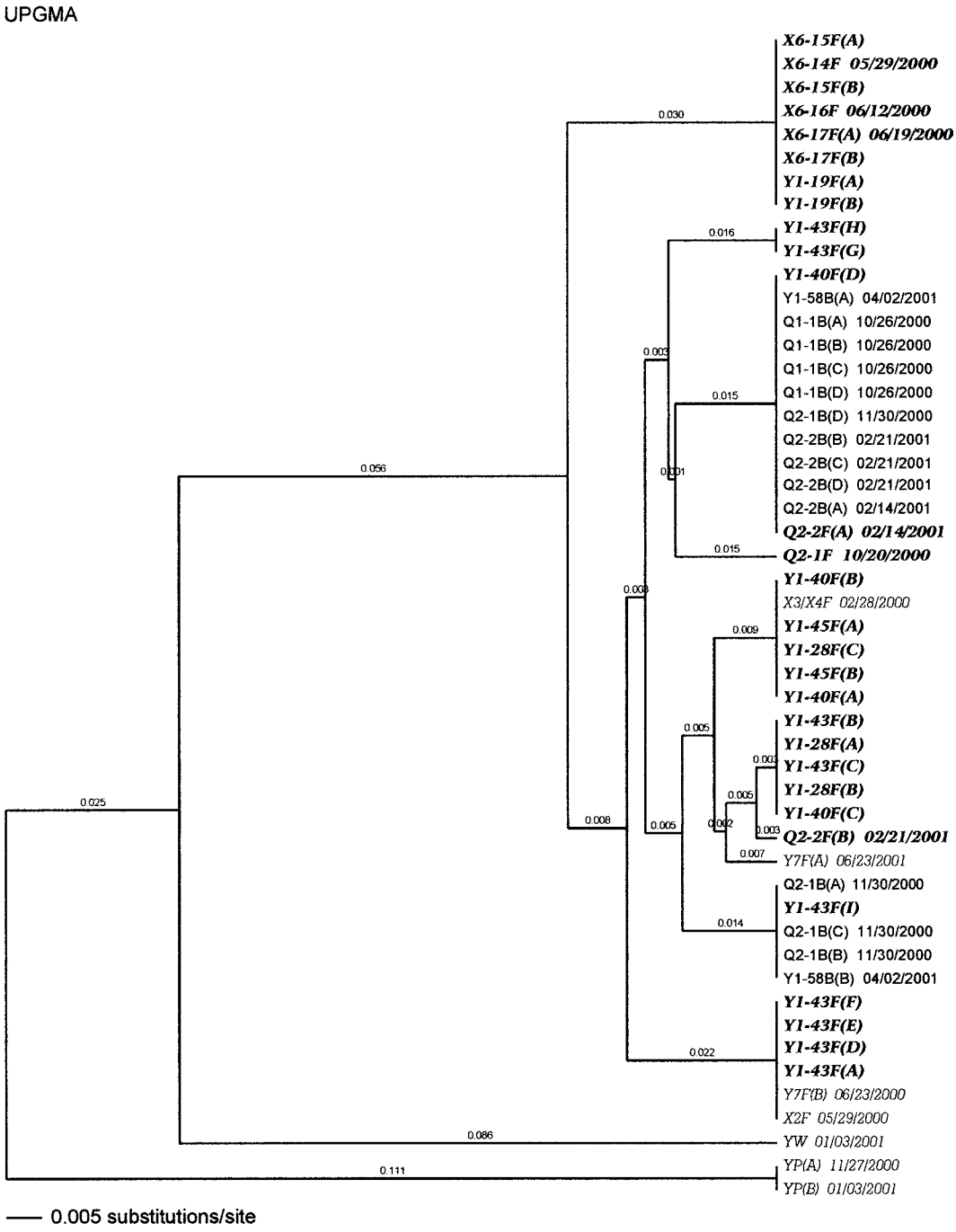

Fig. 1. Relationships derived from comparison of the DNA sequences of the short variable region (SVR) of the *flaA* gene from *Campylobacter* recovered from poultry feces, darkling beetles, and environmental samples. The dendogram was generated using the UPGMA algorithm with HKY85 distance measurements in PAUP*4.0 (Phylogenetic Analysis Using Parsimony). Isolates from distinct sources are delineated using different fonts. The bold italic font represents poultry fecal isolates recovered from the flocks under investigation. The plain font represents isolates originating from beetles. The italic font represents isolates recovered from environmental samples. The date of isolation is also provided.

obtained concomitantly with the beetle isolates was distinct from the beetle isolates.

Three additional environmental samples were positive for *Campylobacter* during the investigation on the breeder production farm Y. These included two pukeko fecal isolates and one creek-water isolate. Molecular subtype analysis demonstrated that the environmental *Campylobacter* isolates were very distinct from the isolates found within the poultry feces and from the beetles sampled from the breeder production farm Y flock.

DISCUSSION

Campylobacter, a foodborne pathogen closely associated with poultry, is considered to be an important agent of human gastroenteritis. Therefore, an understanding of the pathways involved in *Campylobacter* contamination and transmission in poultry flocks is critical for the development of intervention strategies and for the subsequent reduction of *Campylobacter* in poultry. One potential source of *Campylobacter* contamination that is particularly difficult to control is the darkling beetle (*Alphitobius diaperinus*). This investigation sought to provide a better understanding of the link between *Campylobacter* contamination in poultry flocks and the presence of darkling beetles.

The data presented in this investigation demonstrate that multiple clones of *Campylobacter* can be present within a poultry flock. However, the number of clones recovered from beetles was limited. One explanation for the observed decrease in the diversity of *Campylobacter* clones recovered from beetles is that some subtypes of *Campylobacter* may be better adapted for survival in different niches than are others. It may well be that the environmental stresses associated with having a beetle as a niche have led to the preferential survival of specific *Campylobacter* clones. A second possible explanation is that the beetle-associated isolates were recovered using enrichment methodology, whereas the fecal isolates were recovered using direct plating. It has been previously demonstrated that the use of enrichment media for recovery of *Campylobacter* may result in the preferential selection of certain subtypes (23).

Molecular subtype analysis revealed that similar *Campylobacter* subtypes were common to the broiler flocks and to the beetles. Although the temporal cause-and-effect relation of transmission is unclear in this investigation, it could be suggested that the beetles may serve as an ongoing reservoir of contamination. Analysis of *Campylobacter* isolates recovered from the external environment demonstrated varied results. In one of the four instances in which poultry fecal samples [Y7F(B)] from the external environment of the facility were *Campylobacter* positive prior to flock placement, the recovered isolate possessed a *flaA* SVR DNA sequence identical to the fecal isolates of the flock. Alternatively, in three of the four instances, external poultry fecal isolates possessed genotypes that were distantly related to isolates obtained from the related flock. Environmental samples other than poultry feces were rarely *Campylobacter* positive, and when positive, the isolates possessed distantly related clones. These data indicate that the external environment was not a contributing factor to the *Campylobacter* status of the flocks. However, it should be noted that the number of environmental samples obtained in this investigation was quite low. Additionally, the culture methods employed for detection of *Campylobacter* from these environmental samples may not have been adequate for recovery of stressed or injured cells.

One observation of particular interest was the *Campylobacter*-negative status for 14 wk in the breeder flock on farm X. Additionally, once the flock began shedding at week 14 through week 17, only one clone of *Campylobacter* was detected. This particular farm maintained a high level of biosecurity, which indicates that that stringent biosecurity practices, including pest management, may be useful for the control of *Campylobacter* in poultry. Application of similar husbandary practices to broiler production may also be useful in the control of this pathogen. The transmission of *Campylobacter* to poultry is likely to be a complex situation. Improved detection methods and additional epidemiologic investigations are needed to further elucidate the means by which broiler flocks become contaminated with *Campylobacter.*

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